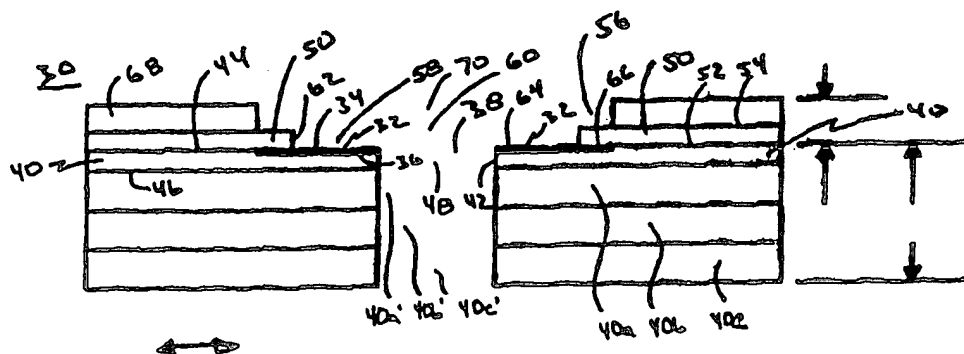




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(54) Title: **LAMINATED ASSEMBLY FOR ACTIVE BIOELECTRONIC DEVICES**



(57) Abstract

Methods of manufacture and devices for performing active biological operations utilize laminated structures (30, 110). In the preferred embodiment, a first planar sample support (50, 72, 104, 122) includes at least one sample through hole (56, 74, 90) a planar electrode (32, 70, 94, 112, 114) is disposed adjacent the first planar sample support (50, 72, 104, 122), and includes an electrode through region (38, 76), a second planar support (40, 78, 96, 102, 124) includes a vent through hole (48, 80, 92, 98), the planar electrode (32, 70, 94, 112, 114) being in a laminated relationship between the first planar sample support (50, 72, 104, 122) and the second planar support (40, 78, 96, 102, 124), further characterized in that the sample through hole (56, 74, 90), electrode through hole (38, 76) and vent through hole (48, 80, 92, 98) are in overlapping arrangement. Preferably, some or all of the through holes, through regions and vent through holes are aligned. In one embodiment, the lateral dimension of the vent through hole is larger than the lateral dimension of the electrode through hole. In an alternative embodiment, the lateral dimension of the sample through hole is larger than the lateral dimension of the vent through hole. In the preferred embodiment, the sample support and planar support are formed of sheet material, most preferably polyimide, having a thickness from substantially 1 to substantially 5 mils. Electrodes are preferably chosen from noble metals, especially gold. Holes or through regions are preferably formed through laser drilling, optionally followed by chemical etching. Interconnect vias provide conductive paths through multiple supports, and are advantageously utilized with hybridized circuitry, especially chip-on flex circuitry.

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DESCRIPTIONLAMINATED ASSEMBLY FOR
ACTIVE BIOELECTRONIC DEVICESField of the Invention

This invention relates to methods of manufacture and devices useful in performing active biological operations. More particularly, the invention relates to devices containing active electrodes especially adapted for electrophoretic transport
5 of nucleic acids, their hybridization and analysis.

Related Application Information

This application is a continuation-in-part application of Application Serial No. 08/534,454, filed September 27, 1995, entitled "Apparatus and Methods for Active Programmable Matrix Devices", which is a continuation-in-part of
10 Application Serial No. 08/304,657, filed September 9, 1994, entitled, as amended, "Molecular Biological Diagnostic Systems Including Electrodes", now allowed, which is a continuation-in-part of Application Serial No. 08/271,882, filed July 7, 1994, entitled, as amended, "Methods for Electronic Stringency Control for Molecular Biological Analysis and Diagnostics", now allowed, which is a continuation-in-
15 part of Application Serial No. 08/146,504, filed November 1, 1993, entitled, as amended, "Active Programmable Electronic Devices for Molecular Biological Analysis and Diagnostics", now allowed, and Application Serial No. 08/709,358, filed September 6, 1996, entitled "Apparatus and Methods for Active Biological Sample Preparation", all incorporated herein by reference as if fully set forth herein.

20 Background of the Invention

Molecular biology comprises a wide variety of techniques for the analysis of nucleic acid and protein. Many of these techniques and procedures form the basis of clinical diagnostic assays and tests. These techniques include nucleic acid hybridization analysis, restriction enzyme analysis, genetic sequence analysis, and the

separation and purification of nucleic acids and proteins (See, e.g., J. Sambrook, E. F. Fritsch, and T. Maniatis, Molecular Cloning: A Laboratory Manual, 2 Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 1989).

Most of these techniques involve carrying out numerous operations (e.g.,
5 pipetting, centrifugations, electrophoresis) on a large number of samples. They are often complex and time consuming, and generally require a high degree of accuracy. Many a technique is limited in its application by a lack of sensitivity, specificity, or reproducibility. For example, these problems have limited many diagnostic applications of nucleic acid hybridization analysis.

10 The complete process for carrying out a DNA hybridization analysis for a genetic or infectious disease is very involved. Broadly speaking, the complete process may be divided into a number of steps and substeps (see Figure 1). In the case of genetic disease diagnosis, the first step involves obtaining the sample (blood or tissue). Depending on the type of sample, various pre-treatments would be carried
15 out. The second step involves disrupting or lysing the cells, which then release the crude DNA material along with other cellular constituents. Generally, several substeps are necessary to remove cell debris and to purify further the crude DNA. At this point several options exist for further processing and analysis. One option involves denaturing the purified sample DNA and carrying out a direct hybridization
20 analysis in one of many formats (dot blot, microbead, microplate, etc.). A second option, called Southern blot hybridization, involves cleaving the DNA with restriction enzymes, separating the DNA fragments on an electrophoretic gel, blotting to a membrane filter, and then hybridizing the blot with specific DNA probe sequences. This procedure effectively reduces the complexity of the genomic DNA sample, and
25 thereby helps to improve the hybridization specificity and sensitivity. Unfortunately, this procedure is long and arduous. A third option is to carry out the polymerase chain reaction (PCR) or other amplification procedure. The PCR procedure amplifies (increases) the number of target DNA sequences relative to non-target sequences. Amplification of target DNA helps to overcome problems related to complexity and sensitivity in genomic DNA analysis. All these procedures are time
30 consuming, relatively complicated, and add significantly to the cost of a diagnostic

test. After these sample preparation and DNA processing steps, the actual hybridization reaction is performed. Finally, detection and data analysis convert the hybridization event into an analytical result.

The steps of sample preparation and processing have typically been performed separate and apart from the other main steps of hybridization and detection and analysis. Indeed, the various substeps comprising sample preparation and DNA processing have often been performed as a discrete operation separate and apart from the other substeps. Considering these substeps in more detail, samples have been obtained through any number of means, such as obtaining of full blood, tissue, or other biological fluid samples. In the case of blood, the sample is processed to remove red blood cells and retain the desired nucleated (white) cells. This process is usually carried out by density gradient centrifugation. Cell disruption or lysis is then carried out on the nucleated cells to release DNA, preferably by the technique of sonication, freeze/thawing, or by addition of lysing reagents. Crude DNA is then separated from the cellular debris by a centrifugation step. Prior to hybridization, double-stranded DNA is denatured into single-stranded form. Denaturation of the double-stranded DNA has generally been performed by the techniques involving heating ($>T_m$), changing salt concentration, addition of base (NaOH), or denaturing reagents (urea, formamide, etc.). Workers have suggested denaturing DNA into its single-stranded form in an electrochemical cell. The theory is stated to be that there is electron transfer to the DNA at the interface of an electrode, which effectively weakens the double-stranded structure and results in separation of the strands. See, generally, Stanley, "DNA Denaturation by an Electric Potential", U.K. patent application 2,247,889 published March 18, 1992.

Nucleic acid hybridization analysis generally involves the detection of a very small number of specific target nucleic acids (DNA or RNA) with an excess of probe DNA, among a relatively large amount of complex non-target nucleic acids. The substeps of DNA complexity reduction in sample preparation have been utilized to help detect low copy numbers (i.e. 10,000 to 100,000) of nucleic acid targets. DNA complexity is overcome to some degree by amplification of target nucleic acid sequences using polymerase chain reaction (PCR). (See, M.A. Innis et al, PCR

Protocols: A Guide to Methods and Applications, Academic Press, 1990). While amplification results in an enormous number of target nucleic acid sequences that improves the subsequent direct probe hybridization step, amplification involves lengthy and cumbersome procedures that typically must be performed on a stand alone basis relative to the other substeps. Substantially complicated and relatively large equipment is required to perform the amplification step.

The actual hybridization reaction represents the most important and central step in the whole process. The hybridization step involves placing the prepared DNA sample in contact with a specific reporter probe, at a set of optimal conditions for hybridization to occur to the target DNA sequence. Hybridization may be performed in any one of a number of formats. For example, multiple sample nucleic acid hybridization analysis has been conducted on a variety of filter and solid support formats (See G. A. Beltz et al., in Methods in Enzymology, Vol. 100, Part B, R. Wu, L. Grossman, K. Moldave, Eds., Academic Press, New York, Chapter 19, pp. 266-308, 1985). One format, the so-called "dot blot" hybridization, involves the non-covalent attachment of target DNAs to filter, which are subsequently hybridized with a radioisotope labeled probe(s). "Dot blot" hybridization gained wide-spread use, and many versions were developed (see M. L. M. Anderson and B. D. Young, in Nucleic Acid Hybridization - A Practical Approach, B. D. Hames and S. J. Higgins, Eds., IRL Press, Washington, D.C. Chapter 4, pp. 73-111, 1985). It has been developed for multiple analysis of genomic mutations (D. Nanibhushan and D. Rabin, in EPA 0228075, July 8, 1987) and for the detection of overlapping clones and the construction of genomic maps (G. A. Evans, in US Patent Number 5,219,726, June 15, 1993).

New techniques are being developed for carrying out multiple sample nucleic acid hybridization analysis on micro-formatted multiplex or matrix devices (e.g., DNA chips) (see M. Barinaga, 253 Science, pp. 1489, 1991; W. Bains, 10 Bio/Technology, pp. 757-758, 1992). These methods usually attach specific DNA sequences to very small specific areas of a solid support, such as micro-wells of a DNA chip. These hybridization formats are micro-scale versions of the conventional "dot blot" and "sandwich" hybridization systems.

The micro-formatted hybridization can be used to carry out "sequencing by hybridization" (SBH) (see M. Barinaga, 253 Science, pp. 1489, 1991; W. Bains, 10 Bio/Technology, pp. 757-758, 1992). SBH makes use of all possible n-nucleotide oligomers (n-mers) to identify n-mers in an unknown DNA sample, which are
5 subsequently aligned by algorithm analysis to produce the DNA sequence (R. Drmanac and R. Crkvenjakov, Yugoslav Patent Application #570/87, 1987; R. Drmanac et al., 4 Genomics, 114, 1989; Strezoska et al., 88 Proc. Natl. Acad. Sci. USA 10089, 1992; and R. Drmanac and R. B. Crkvenjakov, U.S. Patent #5,202,231, April 13, 1993).

10 There are two formats for carrying out SBH. The first format involves creating an array of all possible n-mers on a support, which is then hybridized with the target sequence. The second format involves attaching the target sequence to a support, which is sequentially probed with all possible n-mers. Both formats have the fundamental problems of direct probe hybridizations and additional difficulties
15 related to multiplex hybridizations.

Southern, United Kingdom Patent Application GB 8810400, 1988; E. M. Southern et al., 13 Genomics 1008, 1992, proposed using the first format to analyze or sequence DNA. Southern identified a known single point mutation using PCR amplified genomic DNA. Southern also described a method for synthesizing an
20 array of oligonucleotides on a solid support for SBH. However, Southern did not address how to achieve optimal stringency condition for each oligonucleotide on an array.

Concurrently, Drmanac et al., 260 Science 1649-1652, 1993, used the second format to sequence several short (116 bp) DNA sequences. Target DNAs were
25 attached to membrane supports ("dot blot" format). Each filter was sequentially hybridized with 272 labeled 10-mer and 11-mer oligonucleotides. A wide range of stringency condition was used to achieve specific hybridization for each n-mer probe; washing times varied from 5 minutes to overnight, and temperatures from 0°C to 16°C. Most probes required 3 hours of washing at 16°C. The filters had to
30 be exposed for 2 to 18 hours in order to detect hybridization signals. The overall false positive hybridization rate was 5% in spite of the simple target sequences, the

reduced set of oligomer probes, and the use of the most stringent conditions available.

A variety of methods exist for detection and analysis of the hybridization events. Depending on the reporter group (fluorophore, enzyme, radioisotope, etc.)
5 used to label the DNA probe, detection and analysis are carried out fluorimetrically, colorimetrically, or by autoradiography. By observing and measuring emitted radiation, such as fluorescent radiation or particle emission, information may be obtained about the hybridization events. Even when detection methods have very high intrinsic sensitivity, detection of hybridization events is difficult because of the
10 background presence of non-specifically bound materials. A number of other factors also reduce the sensitivity and selectivity of DNA hybridization assays.

In conventional fluorimetric detection systems, an excitation energy of one wavelength is delivered to the region of interest and energy of a different wavelength is remitted and detected. Large scale systems, generally those having a region of
15 interest of two millimeters or greater, have been manufactured in which the quality of the overall system is not inherently limited by the size requirements of the optical elements or the ability to place them in optical proximity to the region of interest. However, with small geometries, such as those below 2 millimeters, and especially those on the order of 500 microns or less in size of the region of interest, the
20 conventional approaches to fluorimeter design have proved inadequate. Generally, the excitation and emission optical elements must be placed close to the region of interest. Preferably, a focused spot size is relatively small, often requiring sophisticated optical designs. Further, because it is usually desirable to maximize the detectable area, the size of the optical components required to achieve these
25 goals in relation to their distance from the region of interest becomes important, and in many cases, compromises the performance obtained. Accordingly, a need exists for an improved fluorescent detection system.

Attempts have been made to combine certain processing steps or substeps together. For example, various microrobotic systems have been proposed for
30 preparing arrays of DNA probe on a support material. For example, Beattie et al., in The 1992 San Diego Conference: Genetic Recognition, November, 1992, used a

microrobotic system to deposit micro-droplets containing specific DNA sequences into individual microfabricated sample wells on a glass substrate.

Generally, the prior art processes have been extremely labor and time intensive. For example, the PCR amplification process is time consuming and adds
5 cost to the diagnostic assay. Multiple steps requiring human intervention either during the process or between processes is suboptimal in that there is a possibility of contamination and operator error. Further, the use of multiple machines or complicated robotic systems for performing the individual processes is often prohibitive except for the largest laboratories, both in terms of the expense and
10 physical space requirements.

As is apparent from the preceding discussion, numerous attempts have been made to provide effective techniques to conduct multi-step, multiplex molecular biological reactions. However, for the reasons stated above, these techniques are "piece-meal" and limited. These various approaches are not easily combined to form
15 a system which can carry out a complete DNA diagnostic assay. Despite the long-recognized need for such a system, no satisfactory solution has been proposed previously.

Summary of the Invention

Methods of manufacture and apparatus adapted for advantageous use in
20 active electronic devices utilized for biological diagnostics are disclosed. In the preferred embodiment, a multilayer, laminated device includes at least a first planar sample support, the planar sample support including a through hole, a planar electrode adjacent the planar sample support, the electrode including a through region, and a second planar support including a vent through hole, the planar
25 electrode being in a laminated relationship between the first planar sample support and the second planar support, further characterized in that the sample through hole, electrode through region and vent through hole overlap one another. In the preferred embodiment, the planar support members may be formed of a thin, sheet-like material, most preferably a polyamide sheet such as DuPont Kapton™. The
30 preferred thickness of a planar support is in the range from 1 to 5 mils. The planar

sample support material is advantageously selected to have properties consistent with the goals and purposes of the active biological device, for example, exhibiting low binding properties for DNA, having low inherent fluorescence, being relatively inert in an acidic environment and being nonconducting.

5 Stacked or laminated structures may be formed through the use of multiple sheets. In one preferred embodiment, one or more additional layers are disposed above the planar sample support in a direction towards the surface which will receive the biological material. Similarly, a multilayer or laminated structure may be formed beneath the second planar support. The additional laminated layers
10 would typically include through holes, which preferably would be aligned with the remaining through holes or regions. Multilayer or laminated structures may be advantageously formed in numerous configurations. In one embodiment, electrodes may be disposed at differing depths relative to the sample side of the laminated device such that different offset distances between the electrode and the sample
15 applied to the device are achieved. In this way, the different offset distances permit optimization of various functions, such as where complexity reduction and assaying are performed on the same device. Electrodes may be formed at different levels such as through use of an intervening planar support disposed between a first electrode and a second electrode at different levels.

20 In one aspect of this invention, the lateral dimension of the through hole of the first planar sample support is different than the lateral width of the vent through hole of the second planar support. In one embodiment, the lateral dimensions of the vent through hole are larger than the lateral dimensions of the sample through hole. Preferably, a permeation layer is included in the sample through hole at at least a
25 portion of the vent through hole. Relative advantages of this embodiment potentially include the advantageous venting of gas from reactions at or near the planar electrode through a region separate from the hybridization reaction, and the ability of this structure to lock in a permeation layer disposed in the sample through hole, electrode through region and at least a portion of the vent through hole. An
30 alternative embodiment has a sample through hole with a lateral dimension which is

greater than the lateral dimension of the vent through hole. The planar electrode includes at least a portion oriented to face outward through the sample through hole.

In another aspect of this invention, fluidic devices may be formed on, within, or adjacent to the laminated structures. For example, a pump, such as a magnetically driven microminiature pump may be included within the laminated structure. Fluids may be pumped or moved through the system in such a manner.

In yet another aspect of this invention, chip-on flexible circuit technology may be employed to hybridize electronic circuitry in operative contact with the active biological device. Further, multiple level interconnections may be formed, such as through use of vias connecting between one or more layers. In the preferred embodiment, these vias may be exposed to external of the device through the second planar support, rather than through the uppermost planar sample support which adapted to receive biological materials.

The laminated circuit structures and methods of this invention are advantageously utilized in forming active biological devices. A device having a combination of biological complexity reduction and diagnostic assay, as well as counterelectrodes may be formed on a single device.

In yet another aspect of this invention, a multilayer, laminated structure may be utilized to perform biological amplification processes, especially polymerase chain reaction (PCR). Optionally, one or more heaters may be integrated into or formed adjacent the laminated structure to aid in the amplification process.

Accordingly, it is an object of this invention to provide an active biological device having reduced costs of manufacture yet consistent with achieving a small size microlocation.

It is yet another object of this invention to provide a device having a high degree of uniformity of exposed electrode from microlocation to microlocation, as well as device to device.

It is yet a further object of this invention to provide a active biological device having reduced bubbling and reduced burn-out.

It is yet another object of this invention to provide an active biological device having improved gas venting and buffering capacity.

Brief Description of the Drawings

Figs. 1A and 1B show an active, programmable electronic matrix device (APEX) in cross-section (Fig. 1A) and in perspective view (Fig. 1B).

Fig. 2 is a cross-sectional view of a multilayer structure including the electrode in a sample facing orientation.

Fig. 3 is a cross-sectional view of a multilayer structure having a vent hole with a lateral dimension greater than the sample through hole.

Fig. 4 is a cross-sectional view of a multilayer device having a vent hole with a lateral dimension which is greater than the lateral dimension of the sample through hole.

Fig. 5 is a cross-sectional view of a multilayer structure having a sample through hole with a lateral dimension greater than the lateral dimension of the vent through hole, which in turn is larger than the lateral dimension of the electrode through region.

Fig. 6 is a cross-sectional view of a multilayer structure in which the lateral dimension of the sample through hole is greater than the lateral dimension of the electrode through region and vent through hole.

Fig. 7 is a cross-sectional view of a multilayer, laminated structure having a first electrode and second electrode at different distances from the sample surface of the system.

Fig. 8 is a cross-sectional view of a multilayer, laminated structure including an integrated active device, namely, a pump.

Fig. 9 is a plan view of an electrode pattern adapted for complexity reduction and biological assays, including return electrodes.

Fig. 10 is a plan view of patterned planar electrodes and planar sample support for a 3x3 assay array with surrounding return electrodes.

Detailed Description of the Invention

Figs. 1A and 1B illustrate a simplified version of the active programmable electronic matrix hybridization system for use with this invention.

Generally, a substrate 10 supports a matrix or array of electronically addressable microlocations 12. For ease of explanation, the various microlocations in Fig. 1A have been labeled 12A, 12B, 12C and 12D. A permeation layer 14 is disposed above the individual electrodes 12. The permeation layer permits transport of
5 relatively small charged entities through it, but limits the mobility of large charged entities, such as DNA, to keep the large charged entities from easily contacting the electrodes 12 directly during the duration of the test. The permeation layer 14 reduces the electrochemical degradation which would occur in the DNA by direct contact with the electrodes 12, possibility due, in part, to extreme pH resulting from
10 the electrolytic reaction. It further serves to minimize the strong, non-specific adsorption of DNA to electrodes. Attachment regions 16 are disposed upon the permeation layer 14 and provide for specific binding sites for target materials. The attachment regions 16 have been labeled 16A, 16B, 16C and 16D to correspond with the identification of the electrodes 12A-D, respectively.

15 In operation, reservoir 18 comprises that space above the attachment regions 16 that contains the desired, as well as undesired, materials for detection, analysis or use. Charged entities 20, such as charged DNA are located within the reservoir 18. In one aspect of this invention, the active, programmable, matrix system comprises a method for transporting the charged material 20 to any of the specific microlocations
20 12. When activated, a microlocation 12 generates the free field electrophoretic transport of any charged functionalized specific binding entity 20 towards the electrode 12. For example, if the electrode 12A were made positive and the electrode 12D negative, electrophoretic lines of force 22 would run between the electrodes 12A and 12D. The lines of electrophoretic force 22 cause transport of
25 charged binding entities 20 that have a net negative charge toward the positive electrode 12A. Charged materials 20 having a net positive charge move under the electrophoretic force toward the negatively charged electrode 12D. When the net negatively charged binding entity 20 that has been functionalized contacts the attachment layer 16A as a result of its movement under the electrophoretic force, the
30 functionalized specific binding entity 20 becomes covalently attached to the attachment layer 16A.

Fig. 2 is a cross-sectional diagram of a laminated structure 30 according to one embodiment of this invention. An electrode 32 preferably has a generally sheet-like or planar structure at least at certain portions of the electrode 32. The electrode 32 includes an upper surface 34 and lower surface 36. An electrode through region 38 is located in the electrode 32. In the preferred embodiment, the electrode through region 38 is a hole, that is, the electrode 32 completely circumscribes the electrode through region 38. However, the electrode through region 38 need not be formed as a hole, and may only be bounded by or partially surrounded by the electrode 32, or may be set back from the hole as in an annulus.

10 A planar support 40 is preferably formed of a sheet-like material. The planar support 40 includes an upper surface 44 and a lower surface 46, those surfaces generally being parallel to one another. The planar support 40 further includes a through hole 48, defined at least in part by edge 42, also known as a vent through hole in that the through hole 48 is adapted to permit gas, which may form, for
15 example, through electrochemical reactions at or near the electrode 32, to be vented from the laminated structure 30.

A planar support 50 includes a lower surface 52 and an upper surface 54. Again, the planar sample support 50 preferably is of a sheet-like material having lateral extension which is significantly (at least 10:1 times) greater than the thickness
20 of the sample support 50. The planar sample support 50 includes a sample through hole 56, which is preferably continuous around its perimeter.

The electrode 32 is laminated or sandwiched between the planar sample support 50 and the planar support 40. Ideally, the sample through hole 56, electrode through region 38 and vent through hole 48 overlap, and most preferably are aligned
25 when of substantially the same shape and lateral width. The lateral widths refer to measurements in the direction of the two-headed arrow in Fig. 2, namely, within the plane of the sheet. A well 58 is defined by the interior edges 62 of the planar sample support 50, and the upper surface 64 of the electrode 32. Preferably, the interior edge 62 of the planar sample support 50 overlaps at region 66 to form a better seal
30 between the upper surface 34 of the electrode 32 and the lower surface 52 of the planar sample support 50.

Optionally, one or more additional layers may be laminated or otherwise affixed to the structure described previously. For example, additional planar support layers 40a, 40b and 40c may be disposed beneath the planar support 40. Preferably, through holes 40a', 40b' and 40c' are arranged in overlapping relationship with the vent through hole 48, and most preferably aligned thereto. Similarly, one or more additional sample support structures 68 may be disposed on, and preferably laminated to, the planar sample support 50. Again, a sample through hole 70, having a lateral dimension which is greater than or equal to the lateral dimension of the sample through hole 56 is preferred.

10 A permeation layer is disposed within at least the well 58. Optionally, the permeation layer may fill the permeation region 60 which may preferably terminate at the upper surface of the upper most sample support 68, which may also be termed an external sample support in that it provides a surface exposed to the sample materials.

15 The preferred sheet-like material for structures, e.g., the planar support 40 and planar sample support 50 is polyimide. One source for sheet polyimide is DuPont who sells materials generally ranging from 1 to 5 mils thick under the trademark Kapton™. Generally, it is desired that these materials have relatively low swelling (preferably less than 10%, more preferably less than 5% and most preferably less than 2%) in the presence of fluids, preferably have relatively low inherent fluorescence, are substantially inert in an acidic environment (most preferably to a pH of 2 and more preferably to a pH of 1), are electrically insulative or nonconducting. Utilizing currently available materials, relatively thin, e.g., 1 mil thickness sheets, may be patterned with 1 mil wide lines and 1 mil wide spaces.

25 As shown in Fig. 2, multiple sheets may be laminated together to form composite structures. In the exemplary structure of Fig. 2, the planar support 40 and planar sample support 50 are 1 mil thick, the planar supports 40a, 40b and 40c are 5 mils thick and the external contact layer 68 is 2 mils thick. Generally, the use of multiple sample supports 50, 68 above (i.e., towards the side of the laminated structure 30 adapted to receive the sample) a well 58 may be built. As shown in Fig. 30 2, the electrode 32 is at the bottom of a well which is approximately 5 to 6 mils

below the upper surface of the sample support 68 (See the opposed arrows in Fig. 2). Adhesive disposed between the various sample support layers increases the well depth, typically approximately one mil per layer of adhesive. As shown in Fig. 2, the overall laminated structure 30 has a thickness approximately 25 mils (See the
5 oppositely directed arrows in Fig. 2). Laminated structures 30 having thicknesses up to 200 mils or being as thin as 2 mils may be fabricated using conventional technologies.

While polyimide is the preferred material, other materials meeting one or more of the criteria include: polymethylmethacrylate (PMMA),
10 polytetrafluorethylene (PTFE-Teflon), polyester (Mylar), polystyrene, polycarbonate and like materials. Further, various layers in the laminated structure 30 may be selected from different materials to optimize the performance of that layer or the laminate structure 30. For example, the exposed surface of the external sample support 68 may optionally be selected for low adhesion to biological materials. The
15 support 68 may be chosen for its inherent low specific binding with biological materials or the surface of the sample support 68 may be altered to that purpose. One or more layers, especially the external or contact sample support 68 layer may be chosen for high reflectivity, low reflectivity (such as through the use of black or absorbing materials), having a desired texture (e.g., low texture for bonding
20 purposes and surface chemistry optimization), or have hydrophobic or hydrophilic properties. Preferably, the sample support layers, the planar support 40 and the optional sample support 68, are nonporous. The laminated structure 30 is generally preferred to be impermeable to fluids, such as water.

The electrode 32 is preferably formed on or integral to a sheet, such as a
25 polyimide sheet, such as the planar support 40 of Fig. 2. The electrode materials are preferably noble metals, most preferably gold. Generally, it is preferred that no base metals which would adversely affect biological materials to be supplied to the laminated structure 30, such as DNA, are exposed in the electrode 32. Most preferably, it is desirable to avoid copper and iron, and to a lesser extent lead and tin
30 in the materials, or at least, avoiding the exposure of those materials or their ions if present to the biological materials. The electrode 30 should be formed from a

material, and result in a structure, which is generally noncorrosive, is bondable, adheres to other materials, serves to minimize or avoid leakage currents, generates relatively low amounts of electrochemistry and has a relatively high electrochemical voltage at which the surface of the electrode emits constituents materials. Other desirable electrodes may be formed from nichrome, platinum, nickel, stainless steel or indium tin oxide (ITO). ITO being advantageously used when optical detection, especially from the vent side, is utilized. In the preferred embodiment, when polyimide sheets are utilized, the preferred adhesive is DuPont acrylic adhesive, or polyester adhesive. Generally, it is desirable that the adhesive have low squeeze out properties such that during the lamination process, excessive amounts of adhesive do not exit such as at the interior edge 62 of the planar sample support 50, lest excessive, and unpredictable, amounts of adhesive reside on the upper surface 64 of the electrode 32. Generally, the adhesive is on the order of 1 mil. thick.

Fig. 3 is a cross-sectional view of a laminated structure 30 in which the electrode 70 is disposed on the underside, namely, facing away from the side of the laminated structure 30 adapted to receive the sample, on the sample support 72 (or other laminated supports). A sample through hole 74 and electrode through region 76 preferably have the same lateral dimension and are in overlapping, most preferably aligned, relationship. A planar support 78 includes a vent hole 80, again the vent hole 80 being an overlapping relationship, most preferably concentric aligned relationship, with the sample through hole 74 and electrode through region 76. The planar electrode 70 is in a laminated relationship between the sample support 72 and the planar support 78.

Fig. 3 shows the presence of a permeation layer or permeable polymer 82, which was omitted for drawing clarity, though described, in connection with Fig. 2. Additionally, capture probes 84 are disposed on the sample side of the laminated structure 30 at the sample side at the permeable polymer 82.

Fig. 4 shows a cross-sectional view of a laminated structure 30 which differs from Fig. 3 in the thickness of the planar support 78. Whereas planar support 78 in Fig. 3 is relatively thick, preferably at least twice, more preferably three times and most preferably substantially five times, as thick as the sample support 72, the

structure of Fig. 4 has substantially equal thickness of sample support 72 and planar support 78A.

Each of the laminated structures 30 of Fig. 3 and Fig. 4 have a relatively larger volume comprising the vent hole 80 in comparison to the volume of the sample through hole 74. Preferably, the relative sizing of the vent hole 80 to the volume of the sample through hole 74 is selected to reduce gas bubbling and to provide for venting of gas. For example, a volume ratio of 2 to 1, or more preferably 4 to 1, or most preferably 6 to 1 is used. In the embodiments of Fig. 3 and Fig. 4, the relatively larger vent hole volume 80 serves to anchor the permeable polymer or permeation layer 82 within the laminated structure 30. This property is especially advantageous if the permeable polymer or permeation layer 82 swells upon contact with fluids. Further, the structures of Fig. 3 and Fig. 4 have a relatively larger buffering capacity compared to structures not containing that volumetric ratio. Optionally, in the structures of Fig. 3 and Fig. 4, the planar support 78, 78A may be formed of relatively thicker, relatively rigid nonsheet-like material. For example, a laminated 30 may be affixed to another structure, such as a molded flow cell, or other structure formed of acrylic, plastic, metal or the like.

Fig. 5 and Fig. 6 show embodiments in which a laminated structure 30 has a sample through hole 90 which is wider than the lateral width of the vent hole 92. In Fig. 5, the electrode 94 is disposed upon a planar support 96 which has a vent through hole 98 of substantially the same lateral dimension as the electrode through region 100. The lateral dimension of the vent hole 92 in Fig. 5 is greater than the lateral dimensions of the vent hole 98 in the planar support 96 and the electrode through region 100. Fig. 6 utilizes a planar support 102 which is relatively thicker, e.g., twice as thick, as the planar sample support 104.

Fig. 7 is a cross-sectional diagram of a laminated structure 110 in which a first electrode 112 and a second electrode 114 are at different distances from the structure external surface 116 which is adapted to receive a sample. In the embodiment shown in Fig. 7, an intervening planar support layer 118 serves as the offset structure between the first layer electrode 112 and second layer electrode 114. The intervening planar support layer 118 includes intervening through holes 120.

The left most intervening through hole 120 is disposed on the sample side of the laminated structure 110, whereas the intervening through hole 120 on the right hand side is disposed as a vent through hole. A planar sample support 122 is disposed adjacent the intervening planar support layer 118, and sandwiches the first layer electrode 112. The second planar support layer 124 is disposed adjacent the intervening planar support layer 118, having the second layer electrode 114 sandwiched therebetween. As shown in previous figures, probes 126 are disposed on or in the permeation layer 128 which fills at least the sample through hole regions 130, and on the left-hand side of Fig. 7, the intervening through hole 120.

Fig. 8 is a cross-sectional view of a laminated structure 140 which includes a microminiaturized structure 142 disposed on, in or adjacent to the laminated structure 140. Fig. 8 shows a fluidic pump 144 comprising gears 146 shown in meshed relationship. The gears 146 are preferably rotated relative to each other through application of a rotational force, such as supplied by oscillating magnetic fields applied to the magnet disposed within the gears 146. A fluid inlet 148 and fluid outlet 150 provide a fluid path in communication with the fluidic pump 144. An adjacent layer 152 and lateral layers 154 provide containment for the gears 146. The fluid inlet 148 and fluid outlet 150 are defined by the void or space created by supports 156 and exterior layers 158. While a fluidic pump 144 is shown in Fig. 8, other microminiaturized structures 142 consistent with the goals and objects of this invention may be utilized. For example, other microminiaturized structures 142 may include microminiaturized machines, other linear motion devices, valves, actuators, or other micro fluidics components. See, e.g., Dewa, Andy et al. "Design and Implementation of CIGA Fabricated Self-Ringing In-Line Gear Pumps". Solid State Sensor and Actuator Workshop, Hilton Heid, S.C., June 2-6, 1996.

Fig. 9 shows a plan view of a electrode or metallization pattern for a device including a complexity reduction and/or sample preparation region 160, return electrode regions 162 and assay region 164. Traces 166 are shown leaving the various regions 160, 162, 164 for connection external to the device or to other electronic components. The complexity reduction and/or sample preparation region 160 includes traces 166 which include round electrodes 168 having electrode

through regions 170 there through. The return electrodes 162 are connected by trace 166. The assay region 164 has traces 166 which terminate in enlarged electrode regions 172 and have electrode through regions 174 therethrough.

Fig. 10 shows a plan view of a 3x3 array of diagnostic assay sites, surrounded by return electrodes. The array 180 (encircled by dashed lines) shows an underlying trace 182 formed in a circular pattern having an electrode through region 184 therethrough. A planar sample support is disposed above the electrode traces 132 and is shown by the planar sample support interior edge 186. The counterelectrodes 188 have a larger diameter than the assay sites, preferably at least 2:1, more preferably 3:1. The circular electrode 190 terminates at its interior edge in the electrode through region 192. Optionally, the electrode edge may terminate away from the through region of the support, as in an annulus, so as to leave a ring gap of support between the metal and the through hole. Traces 194 connected circular electrode regions 182, 190 to electronic devices or connectors (not shown). Optionally, circuit on flex technology may advantageously permit the positioning of electronic components on the laminated structure 30.

The laminated structures are preferably formed by methods which permit the high yield, low cost manufacturing of high quality devices. The various holes, such as vent holes, sample through holes and electrode through regions may be formed through any known technique consistent with the objects and goals of this invention. For example, microminiaturized drills may form holes as small as 3-8 mils, while laser drilled holes may be as small as 4 mils, or photolithographically patterned holes may be formed to substantially 1 mil. Generally, utilizing current technology, the thinnest sheets permit the formation of the smallest diameter holes. Optionally, chemical etching may be utilized to remove debris from the holes. This technique is particularly advantageous after laser drilling of holes, so as to reduce or remove previously ablated materials. After the electrodes are patterned on the support, and various layers are fabricated, the laminated or composite structure 30 is adhered together. Generally, it is desirable to have minimal or no squeeze out of adhesive to avoid nonuniformity in terms of exposed electrode area. In one embodiment, relatively larger holes are first formed, and then relatively smaller

holes are drilled through the larger holes. Alternately, the supports including vents and holes may be formed first, and then aligned, such as through optical alignment, prior to the setting of the adhesive.

- 5 Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity and understanding, it will be readily apparent to those of ordinary skill in the art in light of the teachings of this invention that certain changes and modifications may be made thereto without departing from the spirit or scope of the appended claims.

We Claim:

1. A device for performing active biological operations comprising:
a first planar sample support including at least one sample through
hole,
5 a planar electrode adjacent to the first planar sample support,
including an electrode through region,
a second planar support including a vent through hole,
characterized in that the planar electrode is in a laminated relationship between the
first planar sample support and the second planar support and in that the sample
10 through hole, electrode through hole and vent through hole are in overlapping
arrangement.
2. The device for performing active biological operations of claim 1
wherein the planar sample support is polyimide.
3. The device for performing active biological operations of claim 1
15 wherein the planar sample support is selected from the group consisting of: PMMA,
Teflon, Mylar, Polystyrene and polycarbonate.
4. The device of claim 1 for performing active biological operations
wherein the planar sample support is from substantially 1 mil to substantially 5 mils
thick.
20
5. The device of claim 1 for performing active biological operations
wherein the electrode is gold.
6. The device of claim 5 for performing active biological operations
wherein the gold is type III A gold.
- 25 7. The device of claim 1 for performing active biological operations
wherein the electrode is nichrome.

8. The device of claim 1 for performing active biological operations wherein the electrode is stainless steel.

9. The device of claim 1 for performing active biological operations wherein the electrode is platinum.

5 10. The device of claim 1 for performing active biological operations wherein the electrode is a noble metal.

11. The device of claim 1 for performing active biological operations wherein the electrode is nickel.

12. The device of claim 1 for performing active biological operations
10 wherein the electrode is an indium tin oxide electrode.

13. The device of claim 1 wherein the lateral dimension of the vent through hole is larger than the lateral dimension of the sample through hole.

14. The device of claim 13 for performing active biological operations wherein the lateral width of the vent through hole is at least twice that of the lateral
15 width of the sample through hole.

15. The device of claim 14 for performing active biological operations wherein the lateral width of the vent through hole is at least three times that of the width of the sample through hole.

16. The device of claim 13 further including a permeation layer disposed
20 within the sample through hole, electrode through region and at least a portion of the vent through hole.

17. The device of claim 1 for performing active biological operations wherein the lateral width of the sample through hole is greater than the lateral width of the vent through hole.

18. The device of claim 1 for performing active biological operations
5 further including an external sample support.

19. The device of claims 1 or 18 for performing active biological operations further including a planar support disposed adjacent to the second planar support.

20. The device of claims 1 or 18 for performing active biological
10 operations in which the sample support has low reflectivity.

21. The device of claims 1 or 18 for performing active biological operations in which the sample support has low fluorescence.

22. The device of claims 1 or 18 for performing active biological operations in which the sample support has hydrophobicity.

23. The device of claims 1 or 18 for performing active biological
15 operations in which the sample support has hydrophilicity.

24. The device of claim 1 further including hybridized circuitry.

25. The device of claim 24 for performing active biological operations wherein the hybridized circuitry utilizes chip-on flex circuitry structure.

26. The device of claim 1 for performing active biological operations
20 wherein the electrode through regions are formed by laser drilling.

27. The device of claim 1 for performing active biological operations wherein the electrode through regions are subject to chemical etching.

28. The device of claim 1 for performing active biological operations wherein the planar electrode includes a trace portion.

5 29. A laminated device for performing active biological operations comprising:

a first planar sample support including at least one sample through hole, the sample through hole having a first lateral dimension,

a planar electrode adjacent the first planar sample support, including
10 an electrode through region,

a second planar support including a vent through hole, the vent through hole having a lateral dimension characterized in that the planar electrode is in laminated relationship between the first planar sample support and the second planar support and in that the sample
15 through hole, electrode through hole and vent through hole are in overlapping arrangement, and in that the lateral dimension of the sample through hole is different from the lateral dimension of the vent through hole.

30. The laminated device of claim 29 for performing active biological operations wherein the lateral dimension of the sample through hole is larger than
20 the lateral dimension of the vent through hole.

31. The laminated device of claim 30 wherein the planar electrode is disposed toward a permeation layer adapted to receive a sample.

32. The laminated device of claim 29 for performing active biological operations wherein the lateral dimension of the sample through hole is less than the
25 lateral dimension of the vent through hole.

33. The laminated device of claim 32 for performing active biological operations further including a permeation layer disposed within the sample through hole, electrode through region and at least a portion of the vent through hole so as to provide a permeation layer located in part beneath the electrode and within the vent
5 region.

34. A multilayer device for performing active biological operations on a sample, the device adapted to receive the sample on a sample surface of the device, comprising:

10 a first planar sample support including at least a sample through hole,
a first planar electrode adjacent the first planar sample support,
including an electrode through region, the first planar electrode being at a first distance from the sample surface,
a second planar electrode, including an electrode through region, the
second planar electrode being at a distance from the sample surface which is
15 greater than that of the first planar electrode, and
an intervening planar support layer serving to support the second planar electrode.

35. The laminated device of claim 34 for performing active biological operations wherein the first planar electrode and second planar electrode are
20 disposed adjacent opposite faces of the intervening planar support.

36. A multilayer, laminated device for performing fluidic operations, the device comprising:

an exterior support layer,
an adjacent layer, disposed in laminated relationship to the exterior support
25 layer,
a second support layer disposed in laminated relationship with the adjacent layer and,
a microminiaturized structure contained within the laminated device.

37. The device of claim 36 for performing fluidic operations wherein the microminiaturized device is a fluidic pump.

38. The device of claim 37 for performing fluidic operations wherein the fluidic pump is a magnetically driven pump.

5 39. The device of claim 38 for performing fluidic operations wherein the magnetically driven pump is a meshed gear pump.

40. The device of claim 37 for performing fluidic operations further including a fluid inlet to the fluidic pump.

41. The device of claim 40 for performing fluidic operations wherein the
10 fluid inlet is defined by a gap between the exterior support and the adjacent layer.

42. The device of claim 40 further including a fluid outlet to the fluidic pump.

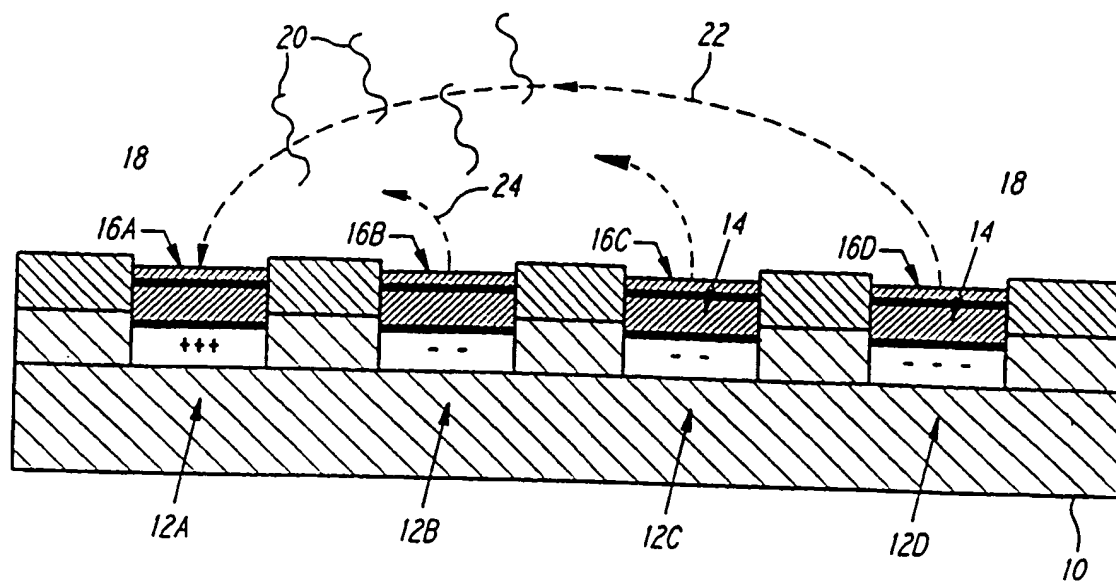


FIG. 1A

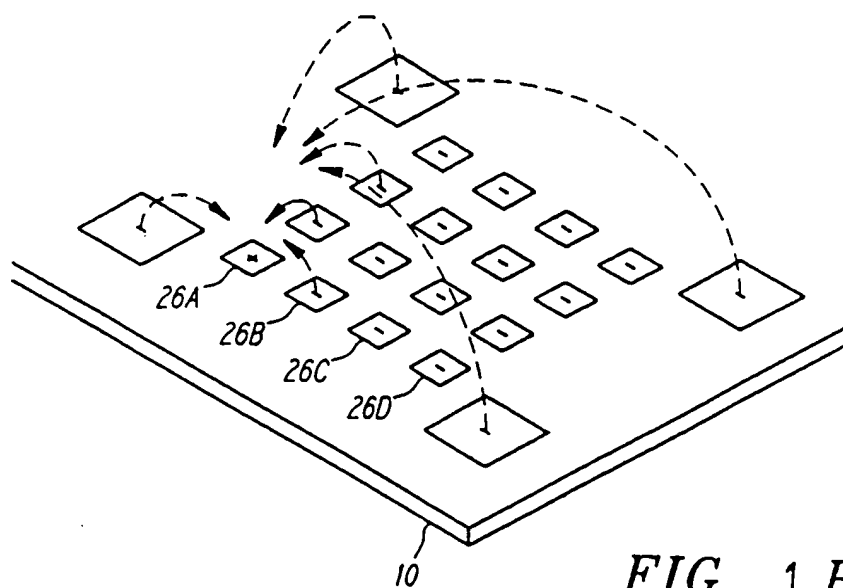


FIG. 1B

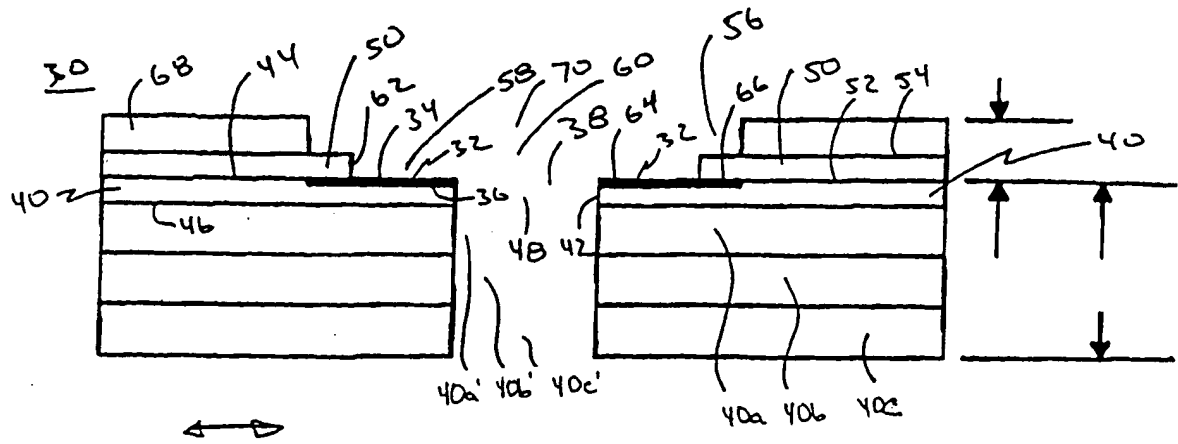


FIG 2

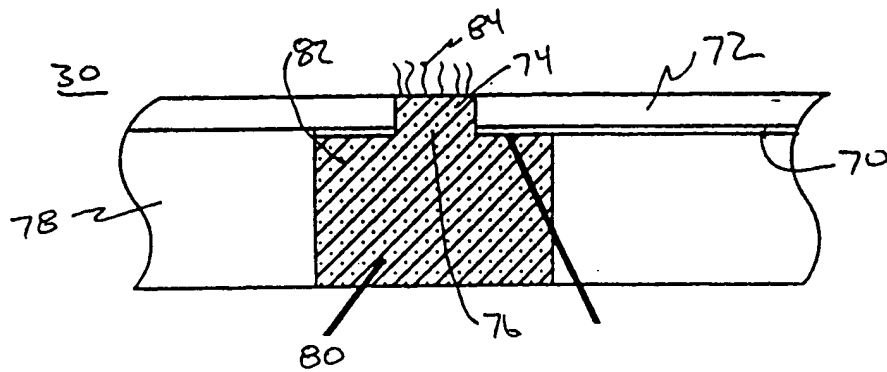


FIG 3

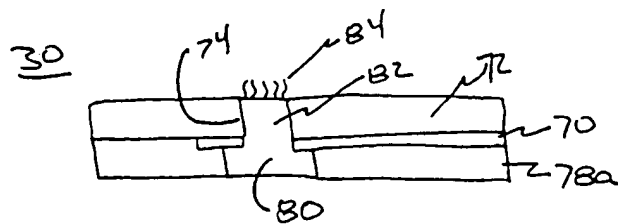


FIG 4

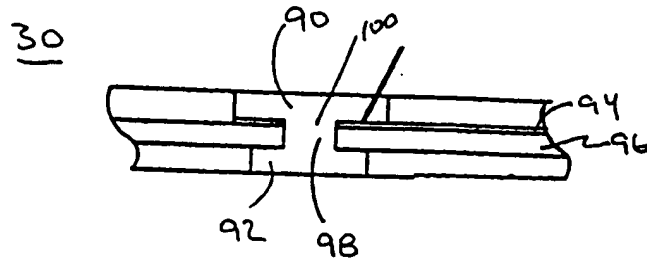


FIG. 5

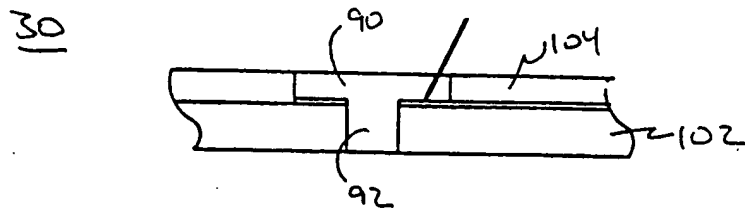


FIG. 6

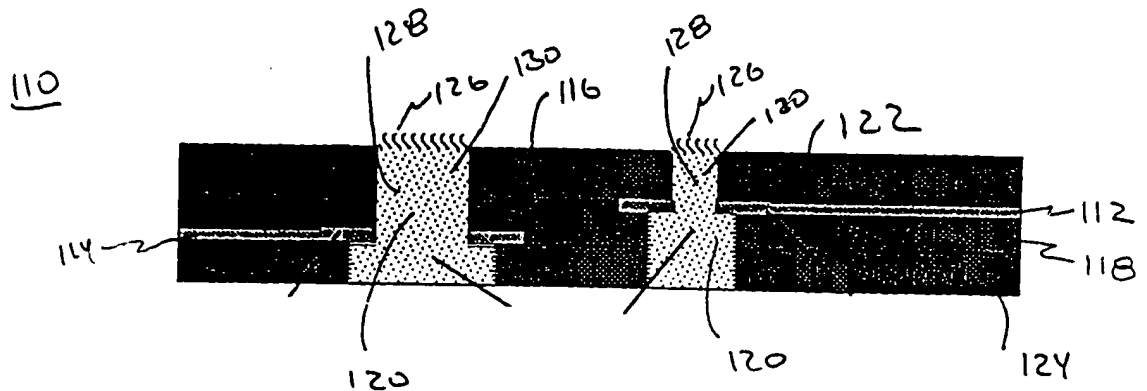


FIG. 7

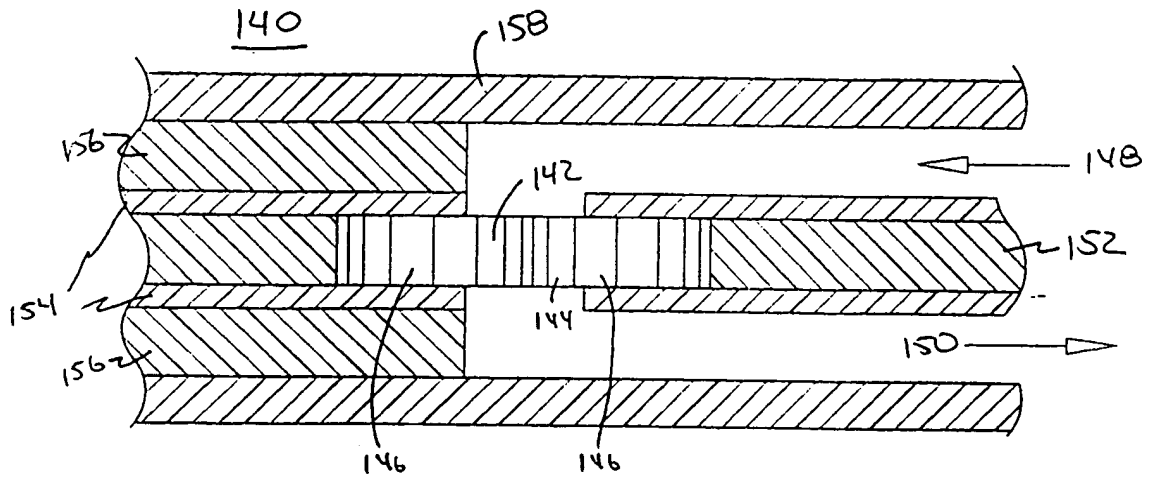


FIG. 8

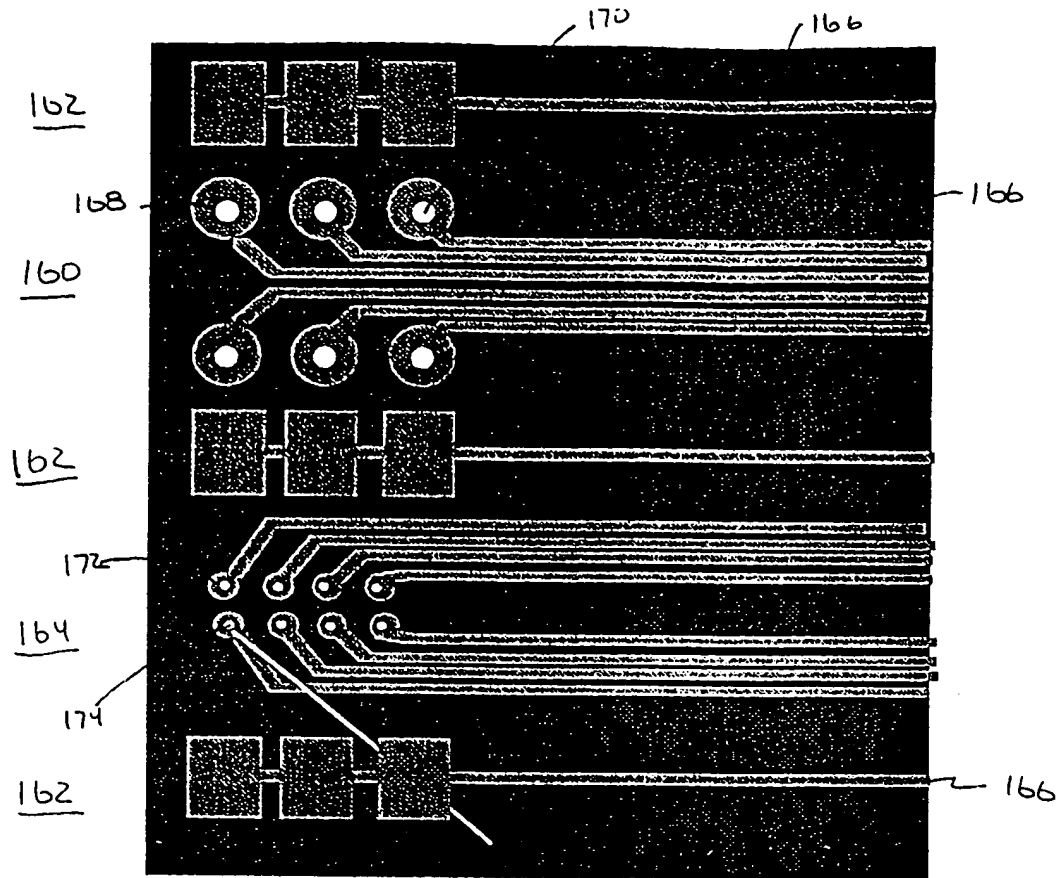


FIG. 9

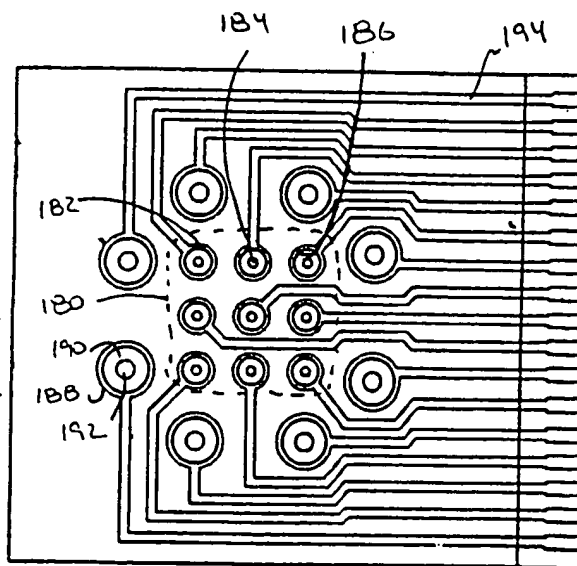


FIG. 10

INTERNATIONAL SEARCH REPORT

Intern. Application No
PCT/US 97/21898

A. CLASSIFICATION OF SUBJECT MATTER IPC 6 B01J19/00 B01L3/00 G01N33/543 F04B43/04		
According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED Minimum documentation searched (classification system followed by classification symbols) IPC 6 B01J B01L G01N F04B		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched		
Electronic data base consulted during the international search (name of data base and, where practical, search terms used)		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	KAZUO SATO ET AL.: "Individual and Mass Operation of Biological Cells using Micromechanical Silicon Devices" SENSORS AND ACTUATORS A., vol. A22, no. 1-3, March 1990, LAUSANNE CH, pages 948-953, XP000371726 see page 949, left-hand column, paragraph 3 - page 950, right-hand column, paragraph 4 see figures 3,5	1.5, 18, 19.27
A	US 5 393 401 A (MEINHARD KNOLL) 28 February 1995 see the whole document	1-35
-/--		
<input checked="" type="checkbox"/> Further documents are listed in the continuation of box C. <input checked="" type="checkbox"/> Patent family members are listed in annex.		
* Special categories of cited documents : <div style="display: flex; justify-content: space-between;"> <div style="width: 45%;"> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> </div> <div style="width: 45%;"> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</p> <p>"&" document member of the same patent family</p> </div> </div>		
Date of the actual completion of the international search <div style="text-align: center; font-weight: bold;">21 April 1998</div>		Date of mailing of the international search report <div style="text-align: center; font-weight: bold;">29/04/1998</div>
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl. Fax: (+31-70) 340-3016		Authorized officer <div style="text-align: center; font-weight: bold;">Stevnsborg, N</div>

INTERNATIONAL SEARCH REPORT

Interr. Application No
PCT/US 97/21898

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 95 01559 A (EVOTEC BIOSYSTEMS GMBH) 12 January 1995 see the whole document ---	1-35
A	WO 96 07917 A (NANOGEN) 14 March 1996 ---	
A	PATENT ABSTRACTS OF JAPAN vol. 18, no. 131 (C-1175), 3 March 1994 -& JP 05 317030 A (HITACHI LTD.), 3 December 1993, see abstract; figures ---	
X	WO 96 14509 A (MICROPUMP CORPORATION) 17 May 1996 see abstract see page 9, line 18 - page 12, line 34 see page 14, line 7 - line 14 see figures -----	36-42

INTERNATIONAL SEARCH REPORT

Information on patent family members

Intern. Application No

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